in the reaction and that would be incorporated into the synthetic nucleic acid in a template-dependent fashion.

Thus, each DNA synthesis reaction yields a mixture of DNA fragments of different lengths corresponding to chain termination wherever the dideoxynucleotide was incorporated in place of the ribo-, 2'-deoxy- or 2'-fluoro- nucleotide. The DNA fragments are labelled, either radioactively or non-radioactively, by one of several methods known in the art and the label(s) may be incorporated into the DNA by extension of a labelled primer, or by incorporation of a labelled ribo-, deoxy-, 2'-fluoro- or 2',3'-dideoxy-nucleotide.

By carrying out DNA synthesis reactions for each of the dideoxynucleotides (ddATP, ddCTP, ddGTP and ddTTP or ddUTP), then separating the products of each reaction in adjacent lanes of a denaturing polyacrylamide gel or in the same lane of the gel if different distinguishable labels are used for each separate reaction, and then detecting those products by one of several radioactive or non-radioactive methods known in the art, the sequence of the DNA template can be read directly. Also, other matrices than polyacrylamide which separate the fragments based on size may be used. Those with skill in the art will also recognize that other nucleotide analogs generally used for reducing sequencing compressions, such as ribo-, deoxy- or 2'-fluoro- nucleoside triphosphates containing 7-deaza-guanine or inosine, may also be used in place of the ribo-, deoxy- or 2'-fluoro-nucleotide for which the respective analog is used.

The present invention also comprises another embodiment of this method for sequencing using 2',3'-ddNTPs and a mutant RNA polymerase which has a reduced discrimination for dNTPs compared to rNTPs. In this embodiment of the method,

the nucleic acid to be sequenced, whether DNA or RNA, is used as a template for *de novo* (i.e., unprimed) *in vitro* nucleic acid synthesis beginning at an RNA polymerase promoter. In this embodiment, a primer is omitted from the reactions and depending on the promoter sequence, in addition to the other components used in the first embodiment, an amount of a dinucleoside tetraphosphate or a trinucleoside penta-phosphate may be added as an initiating nucleotide (Moroney and Piccirilli, 1991) so that the majority of nucleic acid synthesis is initiated from a single site. Because no primer is used, the labelling of the sequencing products must be carried out by one of the other methods envisioned and discussed with respect to the first embodiment of the method or by incorporating a label into or on an initiating dinucleotide or trinucleotide. In other respects, the second embodiment of this sequencing method of the invention is similar to the first embodiment of the method.

The present invention also comprises methods of sequencing nucleic acids by partial ribo-substitution using mutant nucleic acid polymerase which have a reduced discrimination between non-canonical and canonical nucleotides. These methods have advantages over the partial ribo-substitution sequencing method described by Barnes (1977), which relied on the use of a Mn2+-containing reaction buffer to relax the ability of a wild-type DNA polymerase to discriminate between dNTPs and rNTPs. Further, only DNA was used as a template for this method and *de novo* (i.e., unprimed) nucleic acid synthesis was not envisioned. In contrast, the ribo-substitution sequencing method of the present invention uses a mutant nucleic acid polymerase which has an inherent reduced discrimination

between dNTPs and rNTPs and, although it may be included, Mn2+ is not required in the sequencing reactions. In still another embodiment, 2'-fluorine-substituted NTPs are used in place of dNTPs in the ribo-substitution reaction. Embodiments of the method of the present invention also include sequencing using either DNA or RNA as templates and sequencing using either nucleic acid primers or *de novo* nucleic acid synthesis from an RNA polymerase promoter sequence.

Since incorporation of the sequence-delimiting ribonucleotide does not terminate nucleic acid synthesis during partial ribo-substitution sequencing, all of the radioactive or non-radioactive label must be incorporated into the sequencing reaction products prior to incorporation of the first ribonucleotide in order to avoid multiple labeled produced from each nucleic acid molecule synthesized. Multiple labeled products, starting from different positions on the template, would make it difficult or impossible to interpret sequence results. Thus, labeling of nucleic acid products for partial ribo-substitution sequencing must be accomplished by means such as incorporating the label into a primer, when used, or into an initiating di- or tri- nucleotide, or by prelabelling in the presence of an amount of a labeled nucleotide which will be used up or destroyed and/or limiting 2'-deoxy- or 2'-fluoro-nucleotides prior to addition of the sequence-delimiting rNTPs.

We envision sequencing reactions wherein all of the distinguishable non-radioactive labels in, or attached to, or connected with, the products from more than one of the reactions, up to and including all four of the sequencing reactions for a template, or even reactions from multiple